THE CAUSES OF ACCELERATION AND RETARDATION IN THE METAMORPHOSIS OF AMBLYSTOMA TIGRINUM: A PRELIMINARY REPORT.

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DURING the past six seasons the writer has been collecting facts on the metamorphosis of *Amblystoma tigrinum*, both by observation and by experiment. It is hoped to publish an extended account latter; but as relatively definite results have already been reached, the interest of the subject seems to warrant the present publication of an outline, giving conclusions and something of the evidence from which they have been drawn.

The extreme variability of our tiger salamander in regard to the time at which it undergoes metamorphosis is well known. Individuals may breed in the larval condition: witness the Mexican Axolotl, which the concensus of scientific opinion has now practically reduced to the rank of a sexually mature larva of our common species. At least one, and probably two, specimens of our common species, male as well as female, have come into the writer's possession which showed every character of the Mexican animal, even to sexual maturity. More striking than these facts, however, are the anomalies of size which frequently occur. My largest larva weighed, before the beginning of metamorphosis, one hundred and thirty-six grams; my smallest adult weighed but three grams. Thus a larva may outweigh an adult in the ratio of forty-five to one. The above mentioned giant larva, while kept in an aquarium, ate daily, for about two weeks, at least one fair sized adult Amblystoma of its own species; some days it ate two, or followed its cannibalistic meal by devouring a piece of liver the size of a man's thumb.

What are the causes which produce these almost unparalleled extremes of variation? Those familiar with the literature of the subject know that many regard the question as already definitely settled: favorable conditions for aquatic life are supposed to

prolong the larval, gill-bearing stage; while unfavorable conditions for aquatic life — *e.g.*, the drying up of ponds, with forced aerial respiration — are thought to be the regular causes of metamorphosis. Heat and light are regarded by many as subsidiary causes.

This explanation of the metamorphosis of Amblystoma — and of allied forms — as due to a direct response to changing conditions of environment is traditional. It has received further support from casual observations of naturalists, who have seen these animals undergoing metamorphosis on the mud of evaporating ponds. But the final sanction which raised the hypothesis into an almost universally accepted datum of science was Weissman's great article, "On the Change of the Mexican Axolotl to an Amblystoma."[1] This article was based upon Marie von Chauvin's experiments with five larvæ only. Later and much more extensive experiments by the same person were much less favorable to Weissman's conclusions. Indeed a careful study of their methods and results seems to the writer rather to cast doubt upon the entire conclusion that enforced air breathing caused the metamorphosis of these supposed Axolotls. But a consideration of these experiments in detail is beyond the scope of the present article. Brief reference will be made to them latter. I will now pass at once to the results of my own observations and experiments, which have been made on Amblystomæ in the vicinity of Crete, Saline County, Nebraska.

First, metamorphosis, in the writer's vicinity, occurs rarely if at all as the obvious result of enforced air breathing through the drying up of ponds. Diligent search has been made; in one summer over one hundred ponds and larger pools were examined. Many contained larvæ; but none were found in ponds or pools that were less than one foot in depth. The last remnants of large ponds, where larvæ had been abundant a few weeks before, showed no trace of them, although the water might be alive with the tadpoles of the common frog. Experiment showed that Amblystoma larvæ could not usually withstand the temperature of very shallow water exposed to Nebraska sunlight in June, while the tadpoles of Rana were unharmed by it.

[1] A translation of this article will be found in the *Smithsonian Report* for 1877.

However, not death, but early metamorphosis is the probable explanation of this early disappearance. Metamorphosis frequently takes place early in the season, even in June. I have taken small specimens in metamorphosis as early as June from ponds with a depth of water from four to six feet. A careful series of observations, in the case of a single pond, showed the period of wholesale metamorphosis to occur, in this instance, in the latter half of August. Yet in this case unusually heavy August rains had raised the pond to its maximum height and even caused considerable overflow. In spite of repeated search at appropriate times and places, no Amblystomæ have been found in metamorphosis on the mud of drying ponds. That they are so found in other localities the writer knows from childhood experience, as well as from report and record. Our ponds are usually too muddy for direct observation, but the constant use of the dip net has shown me that these larvæ undergo metamorphosis in a considerable depth of water, preferably about three feet. My field notes show but few instances of single specimens in metamorphosis taken from the shallow borders of ponds; many more from deeper water, even up to six feet. In but a single instance have I seen an Amblystoma leaving a pond before the metamorphosis was quite complete.

The second point at which I have failed to make my observations tally with the statements of others is in regard to the habit of rising to the surface for air. It has been stated that rising to the surface for air, or at least the more frequent rising, precedes or ushers in, metamorphosis. I have been favorably situated for the observation of this habit, and have followed its beginnings, its sudden acceleration, its slow or still more sudden cessation, rebeginning, etc.; but whatever the significance of this strange and intricate phenomenon may be, it is certain that, with our larvæ, it stands in no immediate relation to metamorphosis. It may begin and, within a day or two, become incessant with larvæ far too small for metamorphosis, under any conditions. I have seen a group of about a hundred large larvæ, in well aerated clear water, ten feet in depth, so incessantly playing to the surface that the water seemed as if rained upon with large drops at the beginning of a shower. Day after day I found them

equally active in the same place; their gills were long and well fringed; not one showed the labored upward ascent or the dead after-sinking which characterize the larva in which metamorphosis has begun. Suddenly, within twenty-four hours, this air-taking at the surface entirely ceased; a half hour's watching showed hardly a swirl. Yet the larvæ were still there; for a few days later, the water being let from the basin, I caught them, not one showing a trace of gill shrinkage. Experiment with larvæ under controlled conditions conclusively confirmed these general observations. Moreover, larvæ in metamorphosis do not rise with especial frequency; and times of rapid surface play have never coincided with times of wholesale metamorphosis.

It has been further said that, just as the bringing of Amblystoma into the air would force the change to the adult condition, so enforced aquatic conditions would prolong the branchiate condition,— larvæ would not undergo metamorphosis if kept in water and unable to crawl out, *e. g.*, if kept in glass vessels with perpendicular sides. Yet several hundred larvæ have undergone metamorphosis in my laboratory under precisely these conditions; not one, in fact, has refused to do so. The form of the aquarium, the accessibility or inaccessibility of a foothold out of the water have exerted no noticeable accelerating or retarding influence upon the duration of the larval state. Indeed, I have thus far been unable to raise larvæ in the laboratory to their maximum size. I have tried hundreds of them, have varied conditions in many ways, and they thrive well, too well, as the sequel will show; but my largest specimen was but 17.7 cm. in length, while, in one instance, I have seen hundreds of larvæ in a pond that exceeded this dimension. Out-of-door experiments, in tanks, cisterns, etc., have, with one exception, to be mentioned later, led to precisely similar results. Larvæ undergo metamorphosis where the possibilities of terrestrial life are not present; they undergo metamorphosis under conditions where larvæ flourish, but where adults slowly die of starvation.

The most crucial proof, it has been thought, of the power of adaptive response in the young of Amblystoma has been the bringing of the young into shallow water or out of the water altogether, that enforced lung breathing might induce the transi-

tion to the terrestrial form. That animals so treated have, in some cases, metamorphosed is certain; but have sufficient control experiments been conducted to eliminate the possibility of other causes than aerial respiration? The writer has repeated these experiments, and does not find that air-breathing, taken by itself alone, is the natural cause of metamorphosis, or even, in all probability, an accelerating condition. I will instance one set of typical experiments.

Five larvæ, 14 to 18 cm. long, placed on mud with but water enough to partially cover them; refused to eat; kept quiet; all began metamorphosis three days later, and finished eight days later.

Second: Fourteen larvæ, same as above, placed in large aquarium with five inches of water; larvæ quiet; not fed. Began metamorphosis four to five days later, and completed same ten to twelve days later.

Third: Twenty larvæ, same size as above, placed at same time, in aquarium with water just sufficient to cover back fin. Took little food; frequently excited and active. Eight showed signs of metamorphosis in three days. Thirteen at five days. All had completed metamorphosis in eleven days.

Fourth: Twenty larvæ, carefully assorted, with one exception, as to size and other characteristics to match the preceding, were placed in an identical situation; but with a depth of one foot of water. They ate well from the start, the eating habit being established before metamorphosis began. Yet three began metamorphosis on the third day; many on the fifth day. And all, with the exception of the one peculiar, broad-headed specimen, almost simultaneously with those in experiment three.

Fifth. Simultaneously with the above, more than twenty larvæ, not assorted as to size, and mostly smaller than the preceding were placed in a large aquarium supplied with running water. They ate readily. Metamorphosis was more irregular; but on the sixth day many had begun the transition, and it was completed in about the same period as with the foregoing.

Sixth. Above fifty small larvæ from the same source as the above, deemed quite unsuitable for experiment, as they had not reached the minimum size at which metamorphosis commonly

takes place, were placed in the stone basin of a fountain. The food supply was scanty or none; but there was more or less shade and a continual spray of cool water. Here, it was thought growth could be delayed and the larvæ kept for later experiment. Yet on the eighth day the astonishing fact was discovered that many were in metamorphosis. And on the fourteenth day three adults, 9.5, 10 and 11 cm. long respectively, were found. Others had probably escaped; as, despite the perpendicular stone ascent of several inches, it was found they could do.

I have instanced this series of experiments, first, because of all I have conducted, it is the most favorable to the received hypothesis. Second, because it will serve to illustrate further points as well. It will be noticed that there is a slight apparent acceleration in the metamorphosis of the five larvæ placed with their backs out of the water; yet some of those in the aquaria with abundance of water began quite as soon and finished but a day or two later; all that were strictly assorted as to size and other characters followed suit very soon, while still more astonishing is the early metamorphosis of the undersized larvæ in cool well-sprayed water of the fountain. The well-nigh simultaneous metamorphosis of these larvæ so soon after the beginning of these experiments indicates that the chief cause of metamorphosis was alike operative in all cases; what this case was will become obvious later in the discussion. In other instances I have had larvæ prove much more resistent to metamorphosis when kept partially or entirely out of water. I will relate one, which is interesting because it was a semi-natural experiment and, in part, on a large scale.

Around the sloping cement sides of a large artificial reservoir frost had raised a strip of plastering, perhaps a yard in width, leaving a crevice an inch or more in depth below it. Until midsummer this crevice had been several feet below water, and here about two hundred larvæ had formed the habit of concealment, their abraded back fins telling plainly that their occupancy of the crevice had not been temporary. When these had reached a length of ten to thirteen centimetres the water in the reservoir was slowly reduced in depth, during a week or more, until, at first portions, and then the whole of the crevice was above the

water level. The larvæ obstinately refused to leave their accustomed shelter; they could obviously have swum out with the receding water, and in most instances could have crawled or even floundered down the incline into the water, as indeed they did, if the plaster sheets were raised and the larvæ poked a little. I disturbed but few of them however; it seemed an admirable opportunity to watch for enforced metamorphosis. Yet, despite the favorable circumstances that the crevice remained moist and that the larvæ were shielded from the direct rays of the sun, within a few days most of them had died. In only two of the survivors could I find any trace of gill shrinkage sufficient to indicate probable beginnings of metamorphosis. Contrast with this failure to respond to enforced air breathing, the case of ten which, two years before, I had netted from this same reservoir; they had been accustomed to an unlimited supply of well aerated clear water; their branchiæ were unusually long; their whole aspect promised (as I then thought; this being near the beginning of my experience with Amblystoma) a long continuance of the gill-bearing stage. The larvæ were placed on Friday P. M., in a large aquarium with perpendicular glass sides, supplied with abundance of the same tap water in which they had developed from the egg. They were excessively refractory and restless; no attempt was made to feed them. On the following Monday morning the astonishing fact disclosed itself that every larva had undergone metamorphosis; every gill-cleft was closed; the remoulding of head and body shape were complete; only the merest trace of tail fin in a part of the specimens, with gill stubs less than two millimetres in length, were left. Cope, in his study of museum specimens, would have reckoned every one of these specimens as adults. Yet the transformation had taken place from larvæ of an unusually piscine type, within the space of sixty hours; and entirely in water which was abundant, cool and well oxygenated. To return to the case of the larvæ left out of water beneath the sheets of plaster.

Finding, about the fifth day, that most of them were dead, I overturned the loose plaster and secured the last twelve survivors, two of which, as I have said, already showed barely perceptible signs of metamorphosis. All of them were placed on a

moist surface, under large split sponges raised just enough to give the larvæ sufficient room. The sponges were kept wet. In this situation the two larvæ which showed signs of metamorphosis when taken slowly completed the change, and one other, one of the smallest in the lot, after weeks of hardihood in what appeared like a most unnatural and unfortunate existence, underwent still more slowly, an abnormal metamorphosis. The details of this metamorphosis and its final results, both of which I have occasionally had repeated under similar circumstances, are very interesting; but I will not discuss them here further than to say that they appear to me to indicate that metamorphosis by early enforced terrestrial life is wholly unnatural to these animals, and, in a state of nature, would seldom produce an adult capable of survival, even if the metamorphosis was itself successfully passed. The other nine larvæ, taken from beneath the plaster and placed beneath the wet sponges, all died, seven of them without showing any signs of true metamorphosis, although several withstood their terrestrial conditions for many days.

At this point and in connection with the last-mentioned larvæ, I wish to protest against what seems to me a careless and well nigh unpardonable misinterpretation of certain very simple facts in connection with larvæ exposed to air. I refer to the withering of the gill-tips and fringes and the like reduction of the dorsi-ventral fin fold. Again and again in the literature of this subject it is evident that observers and experimenters have looked upon these changes as the natural beginnings of true metamorphosis. That a novice should so consider them is natural enough, but how an observer, broadly and minutely conversant with the facts of amphibian metamorphosis could so interpret them the writer is at a loss to know. The organs in question do of course suffer when exposed to the air, even to moist air; their extreme delicacy makes this inevitable. The seven larvæ, which, in the above trial, died before metamorphosis had begun did take on a somewhat dilapidated appearance before they succumbed. The gills were more or less shrunken at the tips and their fringes partially withered; the back fin, too, had lopped to one side like a wilted plant, and its margin was uneven and somewhat withered. But these changes, high

authority to the contrary notwithstanding, bear no resemblance, and apparently no relation, to the incipient changes of normal metamorphosis, in which gill and fin-fold shrink, always, first at the base, and never at the tip; and in which other fully as important though less obvious changes are never wanting to tell that a vital and inner crisis has arrived. As well interpret the withering comb of the frost-bitten fowl as a sign of metamorphosis as the withering gill tips and fin margin of the air-exposed larva.

It has been claimed, in connection with the hypothesis that aerial respiration is the cause of metamorphosis, that a definite relation exists between gill development and the disposition to retain or discard the larval form. Large-gilled larvæ resist metamorphosis, it has been said; small-gilled larvæ do not, but are easily stimulated to change. Moreover, the claim has been made, that the branchiæ themselves develop more or less fully, according to the amount of oxygen in the water in which they live.

If there is any truth in these two corollaries of the received hypothesis, it is certainly not the whole truth, and the observations of the writer have so far failed to substantiate it. The gills of these animals vary much in size in a state of nature, without obvious relation to the oxygen supply of the water. I estimate that the surface for oxygen exchange varies, in larvæ of equal size, as one to twenty. The causes are by no means clear. In general I find, in confinement at least, that rich nutrition and quiet habits produce the largest branchiæ. The finest specimens, by far, that I have ever seen, in this regard, were two larvæ raised, almost from the egg, in a jar holding but two liters of water which was changed rarely. The branchiæ of these two specimens surpassed considerably, both in length of the rami and in length and number of the fringes, the branchiæ of the finest specimens taken from the deep, clear water of the city reservoir; although this latter source does frequently yield long-gilled specimens. Moreover, these two larvæ, despite their superb branchiæ, the wonder of all who saw them, were, none the less, air breathers; they rose, not infrequently, to the surface for air. And, still more surprising from the ordinary stand-

point, these two larvæ were, one after the other, striken by the sudden impulse to change, the great gills withering almost in a single night; they underwent metamorphosis at about fourteen centimeters in length, in the very jar of water in which they had been flourishing as larvæ so surpassingly well. My aquaria have furnished any number of essentially similar cases. Whether or not it be true that oxygenated water develops the large gill, it certainly cannot be too strongly emphasized that the large gill, when developed, is no impediment to metamorphosis. I once investigated a pond well stocked with thriving larvæ which struck me by the meagreness of gill development; the rami were short, and very thin, the fringes likewise, irregular, few and short. A few weeks later the gills had considerably increased in size and general development. Yet it was following this period of gill expansion that rapid metamorphosis began. The expansion of the gills was here doubtless in no sense the cause of the metamorphosis. They may have been essentially unrelated phenomena. It is possible however that a connection existed as follows: The small-gilled larvæ, although having reached considerable size, had still the delicate larval skin well adapted in itself to subserve the purpose of aquatic respiration. Metamorphosis seldom begins until this larval skin has given place to the more or less thickened integument which is to finally characterize the adult. This change in integument is not here considered as a part of the metamorphosis proper; for the larva may change its integument and yet long retain its aquatic form. Yet this change is a necessary preparation for metamorphosis; and it is quite possible that it should render the skin less effective for respiratory purposes. The cutaneous circulation might even be checked, and a corresponding increase of blood flow to the gills, causing their growth. Certain it is that larvæ with the thickened dermis usually have at least well developed gills, and such larvæ, if the right stimulus comes, are ripe for metamorphosis. I will mention in this connection the surprising fact, developed in the course of several experiments, that the adaptation of these larvæ to entire aquatic respiration stands in no constant relation whatever to branchial development. Many experiments were made to induce metamor-

phosis in larvæ confined strictly to aquatic respiration by nettings, so arranged as to prevent the larvæ from rising to the surface for air. But the surprising difficulty was at once encountered that these larvæ are, all of them, air breathers from a very early stage, and usually cannot, or will not, endure for any length of time complete exclusion from air-taking at the surface of the water. With larvæ of but two centimeters in length, when the development of lungs is but slight, confinement below the surface proved fatal. Even with but two specimens in a large aquarium jar, where they had grown from the egg and thriven in the most natural manner possible, the larvæ repeatedly bored through the netting at night to reach the surface of the water; upon the netting being doubled, one larva still penetrated it, while the other was dead beneath it. This experiment was repeated with many variations in method, but little divergence in result. Larvæ with very large branchiæ, taken from deep, cool, clear water, and introduced singly into a large aquarium freshly filled with the very tap-water in which they had grown would sometimes die of asphyxia in a few hours if prevented from reaching the surface. Such results are very striking. "These animals," said an observer of my experiments, "have gills by the wholesale; but they seem to be mainly for ornament." Not only did the largest gilled specimens succumb when confined beneath the surface of standing water, and sometimes of running water, but they showed no greater ability to live under water than the specimens with the smallest branchiæ that could be chosen. Indeed, when, after many trials, we finally secured two specimens that were indifferent or nearly indifferent to their confinement under running water, neither of them were large-gilled forms, and the one best adapted to this treatment was a very small-gilled specimen. Even specimens in the earlier stages of metamorphosis show little less resistance under this treatment; and in one trial showed even more.

Hand in hand with this inability to live as gill-breathers goes the complete ability to live as lung-breathers, even at very early stages. Seldom, if ever, have my larvæ died of bad or poorly oxygenated water. Over three hundred larvæ, of perhaps two to four centimetres, were, in one instance, transferred directly

from a reservoir where they had grown in pure, deep water, to a single aquarium. Here they lived and throve, although the oxygen of the water was so exhausted that minnows died in a few minutes of asphyxia. Yet the gills of these larvæ remained normal.

If metamorphosis really were caused by enforced aerial respiration, and if loss of branchial surface were a chief factor in the process, both of which are assumed by the common hypothesis, then it would seem to follow as a natural or even necessary conclusion, that cutting off the gills should stimulate to metamorphosis. True, European experimenters on the supposed Axolotl did not find this to be the case; but their failure was explained by the fact that the gills of the Axolotl were quickly reproduced. Besides, the larvæ experimented upon in Europe were resistent to metamorphosis under most conditions. Gill-amputation may have constituted a real stimulus to metamorphosis, and yet one insufficient to bring about the actual change. It seemed to me that our larvæ were much more favorable for the experiment, for they are, most of them, predisposed to early metamorphosis. And, moreover, I have not found that the gills were reproduced with especial facility; weeks may intervene with but little growth, and no specimen in my aquaria has reproduced a gill of normal type; they remain short and truncated.

Yet here again experiment gave practically negative results. At intervals during five days, I removed practically the entire gills from twelve larvæ, a few fringes that were situated on the very gill arches alone remaining. The larvæ were kept in a fair amount of water, which was changed once daily and they fed well. Twelve similar larvæ were treated in a similar manner in every respect except that the gills were not removed. Individuals in both sets soon began metamorphosis, two or three with the cut gills showing a possible acceleration of about twenty-four hours over the earliest specimens in the other lot. This, however, may easily have been accidental, the metamorphosis of the majority in the two lots running an almost exactly parallel course. At the end of the twenty-fourth day, twenty-three out of the twenty-four larvæ, still in the aquaria, had completed the metamorphosis.

One only had proved refractory, not having even begun the change. This was one from which the gills had been removed. Like all the specimens it had thriven and made good growth, yet its gills had grown to barely one third natural size. At this time the specimen was utilized for an experiment under running water, and, despite its several weeks of life with practically no gills, and the small size at which these organs still remained, it withstood fairly well the confinement under water, dying at the end of the second day just after metamorphosis had begun.

The foregoing facts, together with many more like them, have led me to think that the acceleration or retardation of metamorphosis in our species of Amblystoma, is little, if at all, a question of enforced air-breathing, of gill development, of oxygenated or unoxygenated water. Is it then a question of temperature or of light? Again the answer must be largely negative. Not that the writer would deny to these important agencies all influence. A very low temperature checks all life activities in Amphibia; metabolism sinks to the lowest ebb, and metamorphosis is naturally excluded. Light, too, exerts important influences upon the activities of these animals, and thus indirectly if not directly affects growth; and growth and metamorphosis are intimately connected. But abundant observations show that sudden and early metamorphoses are not produced chiefly by excess of light or heat; while delayed metamorphosis is certainly not alone the result of darkness and low temperature. I will mention the following under light:

Larvæ sometimes attain great size in Nebraska ponds despite their complete exposure to the relatively constant sunlight of our clear summer climate. In the laboratory, the largest larva I have ever reared — 17.5 cm. — was kept in a rather small battery jar exposed to the full light of an east window. Others in the same window metamorphosed at very varying stages of growth. On the other hand, larvæ in dark aquaria have frequently metamorphosed with the utmost readiness. In a long series of experiments on the causes of color variation, larvæ were exposed for weeks before metamorphosis to all possible degrees of light and darkness (not to lights of the different primary colors) and no obvious retardation or acceleration of metamorphosis resulted.

Many larvæ have metamorphosed readily after introduction into the almost total darkness of a closely covered, deep cistern.

As to temperature similar facts may be cited; indeed, many of those already cited are obviously applicable. The instance of prolonged larval growth in the east window is especially interesting, for on several of the hottest days of the season other larvæ, in like jars in the same window, died of the heat. On the other hand scores of small larvæ have undergone metamorphosis in shaded aquaria supplied with currents of cool tap water. The instance, already cited, of the phenomenally early metamorphosis of numerous larvæ in the cool water of the fountain basin may be recalled. Even six small larvæ which I introduced into a tank supplied with a stream of the coldest spring water, heavily shaded with trees and covered with two-inch plank, all underwent the change in the course of a few weeks. In short, these Amblystoma larvæ have, with the writer, proved singularly indifferent to wide variations of temperature and luminosity.

What then does control the metamorphosis? But one cardinal factor in the animal's economy is left, and observation and experiment show it to be the dominant factor in question: nutrition. Metamorphosis is a question of nutrition. Stated more accurately, it may be said that metamorphosis is a matter of metabolism, of anabolism passing into sudden katabolism, as the result, usually, of checked nutrition. Other causes may and do coöperate; but a check to nutrition, previously abundant, is by far the most effective and the most frequently operative.

Liability to metamorphosis at any given time is great, in direct proportion to the prevalence of anabolic change at that time; the certainty of metamorphosis at any time is great, in proportion to the suddeness with which anabolism is converted into katabolism. The larva of Amblystoma is an organism, physiologically, in an unstable equilibrium. Carnivorous, an incessant feeder, capable, under the most favorable circumstances, of growing to a length of eighteen centimetres in eight or ten weeks, the overwhelming anabolic changes which are thus maintained tend, when interrupted, to pass suddenly into their opposite, into katabolism. Large larvæ, fed to the maximum, and very fat, lose one quarter to one third of their weight during

a metamorphosis that occupies but a few days. Some constructive changes take place during metamorphosis; but the changes, as a whole, are plainly destructive. The beginnings of the process, the resorption of fin-fold and gills, are plainly processes of self-digestion of peripheral parts. I have seen cases of early enforced metamorphosis, in which it seemed that this destructive action attacked the periphery, not only in fin and gills, but in the legs as well. Now, without for a moment attempting to explain, fundamentally, the nature of metamorphosis, it is thought, none the less, that the description just given makes obvious its intimate relation with nutrition. Looking at the matter thus from the standpoint of physiological facts, and forgetting for the nonce our teleology both "old" and "new," it becomes probable, *a priori*, that quick starvation will be more effective than enforced air breathing in causing the flabby larva to digest its loose and vascular tissues and take on the more compact form of the adult. I may now summarize, briefly, sufficient evidence to support the conclusion that this is the case.

All of the numerous instances observed of extreme acceleration of metamorphosis have been obviously the result of starvation. The three-gram adult, spoken of at the beginning of this paper, was the result of a small larva, accidentally overlooked, and left for several weeks without food, in a jar of water. The excessively early metamorphosis of nearly fifty specimens in the cool water of the fountain is also a case in point, the three or four larvæ that did not metamorphose made little if any growth during the summer, thus showing the absence of available food supply. Metamorphosis in the tank of cold spring water was likewise attributable to this cause; several of the specimens were under the size at which metamorphosis usually takes place; but there was no visible food supply; and the adults that resulted showed very evident emaciation. Many essentially similar instances have been observed.

But prolonged starvation is by no means necessary to stimulate these unstable organisms to sudden change. With full fed larvæ that have reached the length of thirteen to fourteen centimeters a failure to feed for one or two days is almost certain to be followed by a number of cases of metamorphosis. Even over-

eating, followed by indigestion and bloating, frequently upsets the metabolic equilibrium sufficiently to induce the change, and this even in quite small specimens; the accumulation of gas in the digestive tract causes the animal to float for several days, no food is taken, and by the time recovery has occurred the irreversible gill shrinkage, etc., has set in. A careless observer might possibly interpret this floating at the surface, followed by metamorphosis, as an instance where aerial respiration ushered in the change. There is no occasion for such interpretation, however: the animal floats on one side, with the mouth and entire head under water.

Quite in accord with the explanation here given, and constituting a minor confirmation of it, is the fact observed by myself, and independently by an assistant in my laboratory, that in any lot of larvæ of approximately one age it is not the largest specimens that metamorphose first; but an intermediate size. The smallest larvæ will not have finished certain developmental changes which constitute the preparation for the metamorphosis; while the very largest are usually fat, phlegmatic, anabolic larvæ, which are less easily stimulated to sudden katabolic change. Thus in seven experiments, in each of which three larvæ were taken varying somewhat in length, but all of them between eleven and fourteen centimetres, there was but one instance in which the largest of the three began metamorphosis noticeably in advance of its companions, while in four instances the intermediate or the smallest specimen was notably in advance. Much more striking are the facts noticeable in aquaria containing many larvæ, where a few extra large, anabolic individuals frequently postpone metamorphosis for weeks after many of the smaller specimens have become adult.

In close connection with such facts as those just stated and subject to the same explanation is the fact, which I have noted again and again, that the tendency to sudden metamorphosis is directly correlated with the disposition or temperament of these larvæ. Variable as these animals are, in no way do they show greater differences than in their suceptibility to excitement. One of the first ponds well stocked with Amblystoma that I discovered was filled with larvæ of the usual type, but which had

reached a size that is quite unusual in the writer's vicinity. These larvæ were excessively fat, and so tame that, despite the clear water, they could almost be taken in the hand. The specimens removed to the laboratory were equally phlegmatic there, and underwent metamorphosis little if any sooner than their relatives in the pond. The next larvæ discovered in the same summer, likewise in clear water, were so wary as almost to defy my best efforts with the dipnet, and the excitement of those taken was incessant, even during the usually sluggish period of metamorphosis, which, in this case, followed immediately upon their transference to the laboratory. Restless larvæ have, indeed, invariably, with me, undergone metamorphosis as the result of capture and change of conditions. This exceptionally sudden and rapid metamorphosis is no doubt due to the sudden katabolic changes induced by the excessive activity. Even with larvæ of an intermediate degree of excitability, a portion of the individuals usually metamorphose as the result of removal to new quarters, if they have attained the dimensions, etc., which makes metamorphosis easy. And the writer has been able to discover no reason applicable to all such cases, other than that the shock of new conditions, checking or changing food supply a little, always upsets the accustomed rhythm of bodily functions and thereby opens the way for change.

The facts thus far cited have, in the main, been instances of acceleration of metamorphosis, but if their observation and interpretation has been correct, it follows as a natural expectation that facts of the opposite nature should be forthcoming. If a rich food supply suddenly checked is the common cause of early metamorphosis, then a moderate but constant food supply should postpone metamorphosis, until a maximum larval size has been attained, or perhaps postpone it indefinitely. The experimental verification of this side of the proof has been far more difficult, although final success has been reached in a few cases, and these cases quite confirm the view of metamorphosis here adopted. Moreover, the frequent failures are easily explained. As already indicated, our ordinary larvæ grow rapidly, and tend, in the main, to early metamorphosis, *i. e.*, at a length of fourteen centimeters or less. Excepting occasional larvæ of a special type, to be

mentioned later, the only instances of extra-large larvæ which the writer has met with in nature have been instances of larvæ from late deposited ova, in ponds formed late in the season. Such larvæ may live over winter and grow to a very large size — twenty centimeters — by the next June, when metamorphosis occurs. The common explanation would doubtless ascribe this prolonged larval state to the lower temperature of the fall and spring months. But until experiment has given fuller evidence of the influence of temperature, the writer would lay stress on the meagre but constant food supply. For ponds formed late in the season are poorly supplied with Entomostraca and insect larvæ, which constitute the food of aquatic Amblystomæ.

But to return to experimental data. The larvæ worked with thus far have always been rapidly grown specimens; and to furnish them with an even, sparing food supply in the laboratory has not, so far, been successfully accomplished. To keep a constant supply of Entomostraca is not easy; the substitution of earthworms proves far too stimulating, if the supply be regular; and meat, although they thrive on it, is not better: if chopped fine most specimens get none at all, so stupid are they in feeding, and with larger pieces the result is again a too stimulating or a too intermittent diet. The most prolonged larval growth that has been reached, however, has resulted from regular feeding with meat, usually of single specimens in jars. The great point has been to secure the regular taking of food, and, indeed, regular habits all round.

In one instance, two small larvæ were kept the entire summer and fall on *Lemna minor*, a plant which, curiously enough, these carnivorous larvæ will devour in great quantities, although they obstinately refuse algæ and the like. These two specimens (as well as others similarly fed for a shorter time) made no growth at all on their abundant vegetable diet. When my stock of Lemna was exhausted, in the early winter, they refused other vegetable food, until one died of starvation. The other, supplied with meat, grew rapidly, to a length of perhaps eleven or twelve centimeters, when, after an interval in the feeding, it suddenly underwent the same rapid metamorphosis which might, under similar conditions, have befallen it the June before. I instance

this single case to show that mere delay, mere prolongation of the larval period, does not in itself necessarily hinder metamorphosis. Stimulate the flagging life activities and suddenly check the advancing anabolism, and metamorphosis will follow as easily as had growth been rapid from the egg. The opposite opinion has been held by Marie von Chauvin and others.

Laboratory experiments failing, success in rearing larvæ of the largest size was finally reached in cisterns. Although, here too, success only followed after repeated failure. When larvæ of twelve to fourteen centimeters were introduced into cisterns they invariably underwent metamorphosis as a result of the shock and check in food supply. Very small larvæ, introduced in numbers, betook themselves to wholesale cannibalism, resulting in irregular growth and early metamorphosis or death. But larvæ of six or seven centimeters, introduced into cisterns in small numbers, did, in some instances, establish themselves there with interesting results. Several experiments of this kind have been carried out or are now in progress. In one instance, three larvæ withstood metamorphosis for one, two and three years, respectively. The last was taken from the cistern on the fourth summer, still in the complete branchiate condition, although, from faint signs, it is judged that it would probably have become adult during the summer. It was a male and rapidly approaching sexual maturity, the testes showing the first division of the spermatids. These larvæ attained the size of large adults in about sixteen months, growth, in the cistern, being thus only one quarter to one fifth as rapid as the growth of feral or meat-fed specimens. This slow growth indicates that the food supply was light, and the steadiness of the growth, that it was relatively constant.

As a final proof that the metamorphosis of Amblystoma is due to checked nutrition rather than to respiration of air, it seemed advisable to experiment with these larvæ under water, *i. e.*, to subject them to sudden starvation while quite preventing their coming to the surface for air. As the adult Amblystoma frequently passes hours and perhaps days under water, and as observation had shown metamorphosis to take place normally, in the water, why should not the complete change take place

under water? Marie von Chauvin found that the larvæ of *Salamandra maculosa* would not, at least contentedly, retain its gills under water; but her experiments were not carried to their full conclusion.

The attempts to produce the perfect adult Amblystoma under water were not successful, although they fell little short of success. Advanced stages of metamorphosis were produced; in one instance only a trace of tail-fin and gill-stubs remaining. As already indicated, the great difficulty encountered was the unexpected fact that all of the larvæ are air breathers, no matter how complete the development of the branchiæ. No larva could be found capable of living for more than twenty-four hours below the surface of standing water, no matter how pure, or how large the volume. Their tenacity of life varied much. In a few instances, by repeatedly reviving the semi asphyxiated animal in air, or in running water, and replacing it in well aerated water, under the netting, a partial adaptation seemed to be brought about. Fewer movements were made and no distress or impulse toward the surface would be shown for many hours. But gradual or acute suffocation always occurred sooner or latter. When a small stream of water was let fall directly into the aquarium — in this case usually only a battery jar — the results were better; although many did not live long, when situated even thus, and when the stream was checked, even for an hour, our very best water-breathers always made efforts to reach the surface.

Although complete metamorphosis was not reached under water, yet the theoretical importance of the partial metamorphosis which did result is hardly less than would have attached to the completion of the process. The impulse to metamorphose was plainly present. Over thirty larvæ were tried in running water, below netting; and not one was found, of suitable size, that could there resist the tendency to starvation-metamorphosis. All, which did not die of some other cause, began metamorphosis, usually within six days. Sometimes, indeed, the metamorphosis was begun almost immediately, as if the first shock of transference and partial oxygen-starvation were enough, despite the low temperature, to induce the change. Such individuals, did not, however, survive long; those which carried metamorphosis to

completer stages being such as began slowly, after at least several days of contented life below water.

Before leaving these experiments, I will note that, in spite of their fatal results and the convincing testimony they bear to the inability of these organisms to make complete adaptive response to even moderate environmental change, yet they did seem, sometimes, to indicate, some attempt at adaptive response,— in this case an attempt to retain the branchiate condition. For when the fatal resorption of tissues began, the gills were not, as is usually the case, one of the first organs to be attacked. In some instances they showed no diminution in size or function for several days after the back fin was greatly reduced. Such instances also occur in larvæ in ordinary circumstances; but they are not frequent, and may perhaps, in all cases indicate that a high functional activity of the branchiæ is for a time holding in check the katabolic changes which are elsewhere setting in. Similar observations may be made on the occasional overgrown, giant larvæ, in which the branchial apparatus seems frequently to resist metamorphic processes until other parts of the organism are well under way in the change.

A fairly complete résumé has now been presented of the classes of facts which seem to indicate that the metamorphosis of Amblystoma, if not of all tailed amphibia, has heretofore been, to some extent misinterpreted, and that the chief factors in the process are always sudden shifts in metabolism, usually, or at least most easily, induced by changed food supply. It remains to answer one or two queries that naturally arise, and to indicate one narrow group of cases that, at first observation, seem against the present hypothesis.

In the first place, it may very well be asked, if sudden destructive metabolism is the cause of metamorphosis, why should not air breathing favor the change? It would seem to be favorable to rapid oxidation of tissue. The answer is simple: theoretically it should be favorable, and in very rare instances it may actually be so. The writer has known one single instance of three larvæ which showed astonishing activity when accidentally removed from the water, and they underwent the change rapidly. But in all ordinary cases, larvæ, when quite removed from water or

left in water which but partially covers them, become at once inactive. After a few efforts to crawl, and ineffectual turnings about, as if in search of water again, they "settle down, and wait for rain." This inactivity partially compensates the first effects of starvation; so that these air breathing larvæ may metamorphose even less quickly than similar individuals subjected to starvation, but kept in water which encourages movement.

Second: What of the numerous observations on the metamorphosis of Amblystoma in the air, as the result of the drying up of ponds? Do not these, after all, show that metamorphosis frequently follows the enforced use of lungs? They do, indeed, show that metamorphosis follows the enforced use of lungs; but not that it is caused thereby. A moment's thought will show that this naïve, natural interpretation may well be at fault. As the water of a pond evaporates, what is the first result for these larvæ? Plainly a concentration of their food supply. Insect larvæ and entomostraca, moderately abundant before, become now indefinitely easy of access. The larvæ gorge themselves, for a few days, to repletion. Suddenly the last stages of the accelerating evaporation place them at a disadvantage; freedom of movement is checked, or they find themselves partially out of the water. Experiment shows that under such circumstances the larvæ at once cease feeding altogether. The resulting metamorphosis is obvious, and need be due to no other causes than those which the writer has found effective under experimental control.

It may seem more presumptuous to offer a similar explanation for the results obtained by European experimenters on Amblystoma and interpreted by them in so different a manner. Yet a careful reading of a large part of the literature on the subject seems to the writer to inevitably suggest that many of their results are explicable as above suggested. This is especially the case with the series of elaborate experiments made by Marie von Chauvin. Fearing that her charges would die, as indeed they sometimes did, she always prepared them for the trying ordeal of metamorphosis by raising the temperature of the water in which they were kept (to this she ascribes very great importance),

and feeding to the maximum for several days (to which she ascribes no other importance than giving the animals increased strength). The animals were then brought immediately into water sufficiently shallow to force them, for at least part of the time to breathe air. In this latter condition the experimenter complains again and again that it was next to impossible to induce the Axolotls to take any food whatever. Thus in these experiments, too, we have high feeding followed by practical starvation; and it seems that no control experiments were instituted to determine what the effects of over and under nutrition might have been with Axolotls still in an abundance of water. Yet more interesting is it to note that even the varying degrees of success and failure in inducing metamorphosis in these experiments follow closely parallel to varying factors of nutrition, which factors seem to have been wholly neglected in the final interpretation of the results. *Viz.*, as artificial methods of feeding were developed, earthworms being made to crawl down the throats of the refractory larvæ exposed to the air, it became proportionately difficult to induce metamorphosis by withdrawing them from the water. Even for the space of thirteen months, larvæ whose nutritive equilibrium was thus maintained fairly constant, would sometimes retain their aquatic organs despite their exposure to air.

Before closing, brief reference should be made to one class of exceptional cases of retarded metamorphosis which is of great interest, and which might at first thought, seem to contradict some of the considerations set forth in this paper, while offering, however, no support to the ordinary hypothesis. I refer to certain cases of greatly overgrown larvæ, differing in important respects from the ordinary type. It was one of these that I mentioned as a "giant larva," at the beginning of the paper. I hope later to describe this interesting form in detail. At present I will only mention that it is separated from the ordinary type by its much broader and longer head, with parallel instead of converging sides; its enormous gap of mouth; its flattened or usually concave profile, instead of the even curve of the common larva and adult. It has, besides, unusually heavy limbs, with, in most cases, much flattened toes and webbed feet. These larvæ exceed, by several centimetres in length, the size of even the

largest larvæ of the ordinary type which I have as yet seen. The adults resulting from their metamorphosis are nearly as distinct as are the young. They represent, in many features an extreme development of what Cope has designated the "Western form" of *Amblystoma tigrinum*. But, in the writer's vicinity, they seem to constitute only a very small minority of the species, not more than one out of several hundred.

This form, as already indicated, seems to be very resistant to metamorphosis; it has shown itself so under experiment; and, in ponds where the ordinary form leaves the water at a very small size, these occasional giants may continue in the larval state until they have outgrown all but the very largest adult members of the species. There is thus, in the case of these larvæ, plainly another factor present, in the retardation of metamorphosis. And, as both the mature and the immature stages of this form resemble, morphologically, the perennibranchiate types in several respects, it seems, at present, natural to interpret them as reversions toward a more primitive and perhaps perennibranchiate ancestor.

But although these giant, flat-headed larvæ resist metamorphosis without the especial retarding conditions which usually prevent the change, yet it is to be noted that they, too, undergo metamorphosis in the water, and that high feeding, followed by partial feeding or by starvation will apparently invariably bring about the result. I have taken several giants in the early stages of metamorphosis from deep water; and all the specimens in my possession have finally undergone metamorphosis in aquaria. The branchial apparatus seems more resistant than usual; the whole process takes longer; and the order in which the several component changes occur is different; but the physiological aspect of the process, its causes, etc., remain the same, aside from the matter of delay.

A word, in conclusion, as to the significance and setting of these facts in general theory. The writer does not, with Weissmann, hold that the facts relative to the metamorphosis of Amblystoma or the Axolotl may be sufficient to settle cardinal differences of view in biological theory. The conduct of no one species can be conclusive, or, indeed, more than merely

suggestive, however completely it accords with one theory and discredits another. But if the observations and interpretations noted in this report prove correct for all Amblystomæ, it is evident their bearing on the theory of variation is not unimportant. The variations in metamorphosis of the amphibia in general, and especially of Amblystoma, have been looked upon by many as an excellent example of the direct, purposive response of organisms to their environment. Thus, in the Addenda to his work on the *Batrachia of North America*, Cope quotes a writer who deems these animals plastic and responsive in the highest degree,—metamorphosis and remetamorphosis following upon the predominance of aquatic or terrestrial conditions as if the physiological processes of these animals were gifted with both foresight and free will. Marie von Chauvin, at the close of her last article says: "Es sheint den Axolotln eine eminente Befähigung inne zu wohnen, sich den gerade gegebene Lebensbedingungen anzupassen." The writer has no antipathy to the teleological view of variation, providing facts can be found to demonstrate it. Indeed, observations on Amblystoma were undertaken, with the express purpose of giving, if possible, greater precision to the interpretations suggested by Cope and others. But the facts, as here outlined, all tend to support an opposite view. It may even be said that, in the writer's vicinity, the one representative species of Amblystoma survives for no other reason than that, in its larval condition it can appropriate large quantities of food for which there are few competitors. Its other adjustments, instinctive, physiological, and structural, seem very imperfect. The species is indeed plastic; but not purposive, or approximately perfect in its reactions. And in no particular is this more true than in respect to the various structures and structural changes by which these animals are supposed to adapt themselves to aquatic and terrestrial life.

After all, is not this view borne out by the natural history of the Amphibia in general, with the exception, perhaps, of the more progressive, modern type Anura? A half score of forms become lungless in the adult condition,—a degenerative change of doubtful adaptive value. Cryptobranchus and Amphiuma are permanently aquatic, yet without branchiæ, despite their probable

descent from branchiate forms. Siren and Pseudobranchus, although especially aquatic types, exhibit the strangest anomalies of development and retrogression, of use and disuse of gills.

Many forms of Amphibia develop gills in the egg, or in the intrauterine state, which are destined to serve no purpose in aquatic life, even when an aquatic larval state is soon to follow. Of course there may be more adaptation than we know in these anomolous conditions; but do they, in our present state of knowledge, constitute a proof of adaptation or of direct response to environing conditions?

DOANE COLLEGE,
March, 1903.